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A PROCESS FOR IMPRINTING MICRO LAGOON FIELDS IN PLASTIC SURFACES FOR USE IN CELL AND TISSUE CULTURE

by Clarence D. Cone, Jr., and Edward N. Fleenor, Jr.

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SUMMARY

A process for permanently imprinting micro lagoons of practically any desired size, shape, depth, and geometrical array in the surfaces of plastic culturing vessels is described in detail. The process involves the fabrication of a metal impression die possessing a raised image anvil of the micro lagoon field on its surface, and the impressment of the heated die into the surface of suitable plastic vessels. Details of the die fabrication process are given, including the techniques used for photo transference of the basic lagoon-field image to the metal die surface and the development of the anvil imprint image on the die surface by chemical etching. The process used for imprinting the lagoon-field image into the plastic culturing surfaces of various culture vessel types is also described. Photomicrographs of typical lagoon fields formed by the process are presented, along with an analysis of factors in the die-fabrication process which affect the dimensional accuracy of the resulting lagoons.

Time-lapse studies of actual cell growth in lagoons produced by the impression process, using L-strain fibroblasts, indicate that the lagoons are of excellent optical quality and are very effective in preventing both the escape of cells from the lagoons and the intrusion of external cells into the lagoons. The versatility of the fabrication process makes possible the formation of lagoon fields with dimensions specifically tailored for particular applications, two important examples of which are discussed in the paper. The overall process is readily adaptable to mass production of impressed culture vessels and offers a means for making a variety of both general and specific lagoon-field patterns readily available for a wide range of cytological and microbiological applications.

INTRODUCTION

Numerous types of cytological investigations require experiments in which the test subject consists of a monolayer culture of small groups of cells or even of a single isolated cell. In such experiments, it is usually necessary that the test cell or cells be continually monitored and since cells in monolayer culture are highly mobile, the need

arises for preventing the migration of the test specimens out of the field of observation or into areas where they may become lost or unidentifiable through confusion with other cells. Similarly, the intrusion of surrounding cells into the area of the test cells must be prevented. An important example of the need for such micro confinement is in studies utilizing time-lapse cinemicrography, wherein the test cells must be continuously confined, often for long periods of time, to the microscope field of view.

In references 1 and 2, a simple technique is described for accomplishing the physical isolation of cells at the micro level by confining them within tiny ponds or micro lagoons which were formed in a layer of grease or other suitable material deposited upon the primary culturing surface. This technique has proven to be of considerable interest for application in a number of areas of cytological research. The purpose of the present paper is to report the details of a subsequent advancement in the art of making micro lagoons wherein fields of precisely arrayed lagoons of practically any desired shape, size, and depth can be permanently impressed with highly accurate dimensions into the culturing surfaces of a wide variety of plastic culturing vessels and experimental chambers.

The new imprinting technique, although requiring the use of a rather specialized and exacting technology in the fabrication of the impression die, is very simple in application and even with hand-operated impression equipment can be used to imprint rapidly large numbers of culture vessels or chamber windows with lagoon fields of any desired pattern. The impression molding of lagoons offers a number of substantial advantages over the previous grease-layer technique, foremost among these being the permanency of the lagoon field and the exact prescription of any lagoon geometry and array desired. In addition, this method has a number of unique features for specialized cytological applications.

A complete description of the sequential processes utilized in the formation of impressed lagoon fields is presented in this report, including a description of the techniques used to fabricate the impression die, and a description of the manual impression process currently used to imprint plastic-culture-ware surfaces. A discussion is also included of specific cytological uses which have been made of imprinted lagoon fields and of the results of time-lapse studies of the correlation between lagoon depth and containment capability.

DIE FABRICATION AND IMPRINT PROCESSING

This section presents a discussion of the overall process for producing micro lagoon fields in plastic surfaces, including the process for fabricating the lagoon-field imprint die. The fabrication of a die capable of accurately imprinting lagoons of micro dimensions is the key factor in the overall process. The materials used in the fabrication and the source of these materials are indicated in table I. Superscripts on materials (in text) denote number of item in table I. Although these materials were used for this fabrication, any comparable materials may be used as satisfactorily. The impression die is formed by

photographically transferring the exact image of the desired lagoon field to the polished die surface and then chemically etching away the die surface, except for the area covered by the lagoon-field image, until the desired image depth is reached. The photo transference and etching processes are essentially the same as those used for production of micro electronic circuitry for spacecraft applications. After fabrication of the die, imprinting is carried out by attaching the die to the base plate of a precision milling machine, electrically heating it to the proper temperature for the particular plastic being imprinted, and impressing the face into the plastic by using the milling machine spindle and hand-feed control to exert the necessary pressure.

TABLE I.- MATERIALS USED FOR FABRICATION

Material	Source
1. AZ-340 Photo Resist	Shipley Co., Inc.; Newton, Mass.
2. NuArc FT-18A plate maker	NuArc Co., Inc.; Chicago, Ill.
3. AZ-300 developer	Shipley Co., Inc.; Newton, Mass.
4. PEMCO vertical rotating spray etcher	Pesek Engineering and Manufacturing Co.; Minneapolis, Minn.
5. Hi-speed circuit etch	Philip A. Hunt Chemical Corp.; Palisades Park, N. J.
6. Variac rheostats	General Radio Co.; West Concord, Mass.
7. Falcon plastic culture ware	Falcon Plastics; Los Angeles, Calif.
8. Polyvinyl plastic slides	Carolina Biological Supply Co.; Burlington, N. C.

Fabrication of Imprint Die

Preparation and polishing of initial die surface.- A dimensioned drawing of a typical die blank is shown in figure 1. It consists of a cylindrical base of yellow brass, 2.0 inches (5.08 cm) in diameter by 5/8 inch (1.59 cm) thick, and containing a 1/8-inch-diameter (0.32 cm) cylinder bore for insertion of the die heating element. The brass base is surfaced by soldering to one end a 2.0-inch-diameter (5.08 cm) disk of 0.030-inch-thick (0.08 cm) beryllium-copper alloy. The die-blank dimensions given are for a specific die designed to imprint a square array of lagoons in the bottoms of 70-mm-diameter plastic Petri dishes. In general, however, the dimensions of the die blank can be variable, depending upon the size of the lagoon-field image and the dimensions of the culturing vessel or coverslip into which the image is to be pressed.

The beryllium-copper surface is subsequently polished to an essentially mirror finish (4 microinches (0.9 nm) finish) by a series of graded lappings, proceeding successively through 320- and 600-grit carborundum, polishing cotton, and cerium oxide

glass-polishing treatments. The polished surface is finally degreased in preparation for image transference by ultrasonic cleaning in a detergent solution, followed by water rinsing.

After final polishing, it is essential in all subsequent operations to protect the die surface from sources of scratches if good optical quality is to be obtained in the final impressed lagoons.

Photo transference of the lagoon-field image.- The next step in the process involves the photographic transfer of the lagoon-field image to the die surface, using an $FeCl_3$-etchant resistant material to form the image. This transfer is accomplished by first coating the polished, degreased surface with a thin film of the etchant-resistant material [AZ-340 Photo Resist][1], which becomes soluble upon exposure to ultraviolet light and subsequent chemical development. The die surface is dipped into the filtered etchant-resistant solution and then spun for 4 minutes at 1250 revolutions per minute to remove the excess fluid and to insure an even coating of the surface. The die is then transferred to a hot-air oven and maintained at 140° F for 3 hours to allow drying of the surface coating. The entire coating and drying operation must be carried out under ultraviolet-free conditions.

An ×20-scale drawing of the final desired size of the lagoon-field pattern is accurately prepared in India ink and photographically reproduced on high-contrast positive film at a 1/20 reduction. This film pattern is then placed with its emulsion side down against the Resist-coated die surface, and the ensemble exposed in vacuo (at 5 to 7 mm Hg absolute pressure) to a source of ultraviolet light [NuArc FT-18A plate maker][2] for 4.5 minutes. After exposure, the die is placed in a developer solution [AZ-300 Photo Resist developer][3] for 2.5 minutes, removed, rinsed in cold water, and air dried. The die is subsequently heated to 140° F for 1 hour to dry and harden the surface image of the lagoon field. The etchant-resistant image is clearly visible at this stage.

Lagoon-field image engravement by chemical etching.- For physical engravement of the lagoon-field image into the die surface, the die is placed in a spray etching chamber [PEMCO Vertical Rotating 18-inch by 18-inch spray etcher][4] and exposed to the $FeCl_3$ etchant spray for a length of time sufficient to give the desired image-anvil height. The etchant used [Hunt's Hi-Speed circuit etch][5] will remove approximately 30.5 microns of copper per minute from the die surface at 100° F. The etchant chemically reacts with and solubilizes all areas of the die surface except those of the image protected by the Resist coating, and thus produces a raised projection of the image, referred to herein as the image anvil. Upon completion of the etching treatment, the die is removed from the chamber, thoroughly rinsed in cold water, and dried. Finally, the resist coating remaining on the image-anvil surface is removed by rinsing the die in pure acetone and drying; the die is then ready for use in imprinting the micro lagoon pattern into plastic.

Factors affecting accuracy of image engravement.- A typical completed imprint die showing the image anvil of a square array of circular lagoons of graded sizes is illustrated in figure 2. Each horizontal row of anvil spikes produces 5 lagoons of identical size and depth upon imprinting. The actual lagoon diameter associated with each row for this die is given in table II; all lagoons, by the nature of the etching process, necessarily have the same depth. The image anvil of the die shown in figure 2 has a height of 45 microns and produces lagoons of the same depth.

TABLE II.- ACTUAL LAGOON SIZES

Row	Lagoon diameter, microns
1	30
2	195
3	450
4	710
5	960

Figure 3 presents a series of photomicrographs of representative anvil spikes (representing individual lagoons in the final impression) from the die of figure 2. Stereomicroscopic study of such spikes reveals that they have the geometrical form of truncated cones. This shape is to be expected from the nature of the etching process used in their formation, as can be seen by reference to figure 4. Since the etchant attacks the metal surface wherever it is exposed, as soon as the tips of the anvil spikes have begun to form because of the vertical removal of metal, the etchant begins to eat away metal of the spike sides in a lateral direction (that is, parallel to the die surface), and thus reduces the tip (or face) diameter of the spike. Since it requires some time (approximately 1.5 minutes) for the etchant to remove the horizontal-surface metal to the desired depth, the parts of the spike sides which were formed first (that is, the side regions nearest the tip) are in contact with the etchant for a longer time than those nearest the base, and the resulting increase in metal removal at the tip produces the conical form. The cone half-angle of the spikes shown in figure 3 is approximately 30°; thus, the rate at which the etchant (used in forming this die) removes metal in the vertical direction (that is, in the direction of the spray, normal to the basic die surface) is $\sqrt{3}$ or 1.73 times the rate of removal in the lateral direction. The action of the etchant in the formation of truncated conical spikes in the present case is precisely analogous to the situation existing in the electrolytic formation of ultrafine metal microelectrode tips by axial oscillation of fine wires in the electrolyte. (See ref. 3.)

This lateral etching action has an adverse effect on the accuracy of the reproduction of a given photographic image. The lateral removal of metal reduces the actual tip

diameter of the anvil spike below that of the photographic image, and thus results in a smaller imprinted lagoon than intended. This error becomes increasingly more pronounced as the lagoon image size is decreased, since the set amount by which the etchant progresses laterally at the spike tip for a given spike height becomes an increasingly larger percentage of the basic image diameter. Thus, for example, a photographic lagoon image of 100-micron diameter, when etched for a sufficient time to produce an anvil spike 45 microns high, will result in a spike tip diameter of only about 48 microns, the vertical-to-lateral etching ratio being 1.73. The degree of reduction in final lagoon diameter produced by this effect depends, of course, upon the ultimate lagoon depth desired, since the longer etching times required to obtain greater vertical depth also produce additional lateral removal of metal at the tip. This effect can be easily overcome, however, by making the photographic images of very small lagoons sufficiently oversize to compensate for the lateral etching action. (See fig. 5.)

Imprinting Process

Die heating and impression procedure.- The general prototype arrangement presently used for manual imprinting of lagoon-field images into plastic culturing surfaces for use in the molecular biophysics laboratory (MBL) at the Langley Research Center is shown in figure 6. The die is mounted face upward on a sheet of methyl methacrylate plastic (which serves as a thermal insulator) and secured to the base plate of a precision milling machine. A similar cylindrical, smoothly polished pressplate is mounted on the milling machine spindle (see fig. 7) and used to apply the impression pressure through movement of the machine's hand-feed control. Both the basic die and the pressplate are provided with simple heating elements consisting of common fine-tipped soldering irons (fig. 8) whose heat output is regulated by separate rheostats [Variac][6] (fig. 6).

For lagoon-field impression, both the die and pressplate are heated to the proper temperature and the plastic vessel or surface blank is placed on the die surface. (See fig. 9.) The dish bottom being imprinted in figure 9 is used subsequently for preparing a coverslip for use as a perfusion chamber window. For imprinting of the inside bottoms of plastic Petri dishes, the heating element of the die is removed after the die has reached the proper temperature, and the dish placed over the die as shown in figure 10. Approximately 15 seconds are allowed for the plastic to preheat in order to evaporate all surface moisture, after which the heated pressplate is brought down with sufficient pressure to insure full penetration of the image anvil into the plastic. It has been found through experience that although pressure alone with a cold die is often sufficient to make an impression in most plastics, some degree of heating greatly increases the clarity and sharpness of the impressed image, and hence is highly desirable. Unless the plastic to be impressed with a heated die is preheated sufficiently, there is a tendency for small bubbles (probably

of water vapor) to form in the region of the impression and produce severe optical distortions in the lagoons. Spindle pressure is maintained for approximately 20 seconds. (See fig. 9.) The pressure is then released, and the finished imprinted vessel removed from the press is ready for sterilization and use.

Plastics used for lagoon-field impression.- Most plastics used for culturing of cells and other microbiological subjects are easily imprinted with micro lagoon patterns. One example of a suitable plastic is that used in plastic culture ware such as Petri and Cooper dishes [Falcon plastic culture ware][7]. Experiments have shown that a die temperature of 180° F makes excellent lagoons in this plastic and that cells grow as readily on the impressed lagoon surfaces as on the basic culture surface. A second example is polyvinyl plastic of the type used for microscopic slides [Carolina Biological polyvinyl plastic slides][8]; this material imprints well with die temperatures of only 120° F.

Examples of typical lagoons.- A lagoon-field array which has been found highly useful at the MBL is that pictured in figure 2. This field provides an assortment of useful lagoon sizes and the square array permits rapid location and identification of any particular lagoon by use of the row-column designation system. The connecting-line grid of this pattern is also very helpful in rapid scanning across a row or column of lagoons.

Figure 11 presents a series of low-magnification photomicrographs of three representative lagoons made with the die shown in figure 2, and figure 12 shows higher magnification micrographs of two typical lagoons made with this same die. The lagoons are 45 microns in depth.

CYTOLOGICAL USES OF IMPRINTED LAGOON FIELDS

Lagoon Inoculation and Cell Growth

The various procedures which can be used for inoculating the impressed lagoons are the same as those described in reference 2 for the case of the grease lagoons. The cells can simply be allowed to settle from suspension, with chance population of the lagoons, or a given lagoon can be directly inoculated with a particular cell or cells by use of a micromanipulator. The inoculation operation in the latter case is much simpler with the impressed than with the grease lagoons, since the impressed lagoons are very rigid and not subject to easy damage or complete destruction by accidental contact with the inoculating pipet.

Cells, in general, grow and proliferate well in the micro lagoons formed by imprinting. Surface attachment of cells appears to be just as firm on the lagoon surfaces as on the basic culturing surface in which they are impressed. Figure 13 presents a series of representative micrographs of L-strain fibroblasts growing in circular lagoons of various sizes.

Lagoon Depth and Cell Escape

To determine the dependency of the confinement and isolation capabilities of impressed lagoons on the lagoon depth, a series of patterns of circular lagoons having identical geometrical forms (the same as those shown in fig. 2) except for lagoon depth were tested. Lagoon fields having depths of 10, 40, and 60 microns were examined, the fields being impressed in the bottoms of Falcon Petri dishes which were subsequently removed and used as windows of the MBL perfusion chambers. (See ref. 4.) For these tests, the fields were inoculated with L-strain fibroblasts and followed in time-lapse cinephotography for up to 96 hours. These cells make excellent subjects for such escape tests, since they are highly mobile and have been found in previous work with grease lagoons to possess an exceptional tendency to escape from shallow lagoons.

The results of these tests indicate that reasonably effective confinement and isolation are obtained with the L-strain for lagoon depths of 40 microns and greater; however, the fibroblasts escape readily from the shallow lagoons (10-micron depth). In regard to intrusion of external cells into the lagoons, the same results apply; there are relatively few intrusions for the deeper lagoons (40- and 60-micron depths), whereas intrusions occur readily for the shallow lagoons (10-micron depth) if the cell concentration surrounding the lagoon is moderately dense. In the case of sparse cell concentrations (or densities) on the surrounding surface, intrusions are very seldom seen, even for shallow lagoons.

The primary factor in preventing cell escape from the deeper impressed lagoons appears to be the relatively sharp (approximately 60^{o}) corner formed where the lagoon bottom surface intersects the almost vertical walls of the lagoon. Cell pseudopods appear to retract upon contact with the vertical walls of the lagoon and thus the cell remains confined to the lagoon interior. This pseudopod retraction appears to be independent of chamber orientation with regard to gravity; that is, whether the lagoon surface is oriented so that gravity is tending to pull the cell from the surface or to pull it to the surface. (See ref. 2.) Prevention of cell intrusion seems to result from the same phenomenon, namely, the relative sharpness of the intersection of the lagoon walls with the primary culturing surface to which the cells are attached. In general, this "bend" is not as sharp as that at the lagoon bottom but it is apparently steep enough to ensure that the cells do not readily negotiate it. The surface texture of the die in this bend region is relatively coarse, and this texture may also have some effect in preventing cell movement over this surface. Cells can often be seen alined along the outer edge of this region, but seldom cross it.

In describing the confinement and isolation properties of the impressed lagoons as "reasonably effective," it is not meant to infer that escapes or intrusions never occur; however, they occur so infrequently for the deep lagoons that many practical tests requiring cellular isolation or confinement for periods up to several days can be successfully carried out.

Specific Lagoon Forms and Uses

Basic lagoon-field patterns.- Two basic lagoon-field patterns which have been found useful for general research purposes are pictured in figure 14. The first is essentially the same as the square array shown in figure 2, whereas the second consists of a radial-circumferential array of 40 circular lagoons consisting of 8 lagoons each of 5 graded sizes. When centered on a rotating stage, each circumferential "row" of this field can be quickly scanned by simple rotation from one lagoon to the next. Although these two simple patterns of lagoons are convenient for general use, they are but two representatives of a wide range of possible forms for which imprint dies can be readily fabricated. As is obvious from the foregoing description of the die fabrication process, practically any desired form of lagoon field for which a suitable drawing can be made can be imprinted in plastic vessels.

Specific culture vessels.- Three basic culture vessels utilizing micro lagoon fields have been used for research purposes at the MBL. Two of these, the plastic Petri and Cooper dishes, are shown in figure 15, with the square lagoon-field array imprinted. The imprint is made directly in the bottom of the Petri dish and viewing is conventionally carried out by using an inverted microscope, although an upright microscope can be used if long working-distance objectives are available. Although the letter-number system of a Petri dish lagoon-field imprint intended for viewing by an inverted microscope will be reversed when viewed with an upright microscope, the reversal presents no real problem in lagoon identification and the same imprint die can be used for both types of imprints; otherwise separate dies, one with reversed designations, must be used. For the Cooper dish, the imprint is made on the underside (immersion) surface of the cover and the lagoons can be viewed with an upright microscope. The same criteria of designation reversal and viewing direction as discussed above for the Petri dish also apply for the Cooper dish. Methods for inoculating the Cooper dish cover are given in reference 2. The third type of vessel is the MBL perfusion chamber described in reference 4. The lagoon field in this case is imprinted on a plastic coverslip which serves as the chamber window, and viewing can be done with either the upright or inverted microscope. In general, the thinner the plastic into which the lagoon field is impressed, the better the resulting optical qualities of the lagoons for microscopic viewing.

Specialized uses.- In addition to general research uses of micro lagoon fields such as pictured in figure 14, the versatility and simplicity of the die fabrication make possible the impression of precisely "tailored" fields for a number of specialized uses, two of which are mentioned here. The first involves the formation of lines of "trenches" in Petri dish bottoms for use in holding free spherical cells stationary during micromanipulation operations such as, for example, impalement of microelectrodes in measurements of membrane potential. For this purpose, the cells are positioned in micro trenches having widths slightly less than the cell diameter and a depth about equal to the cell radius,

and are thus held securely while impalement is made through the upper surface. The second use involves the formation of rectangular micro lagoons for use in time-lapse studies, the lagoon dimensions being precisely tailored to fit the time-lapse framing size. The lagoons are dimensioned so that they exactly fill the film frame for the particular magnification being used and thus provide an isolation and confinement boundary around the test field, but still permit use of the entire frame area for cell observation.

CONCLUDING REMARKS

This paper has described in considerable detail a process for imprinting in plastic surfaces micro lagoon fields suitable for use in a wide range of cytological applications. Although the imprinting process itself is very straightforward, the fabrication of the requisite impression die does require the use of rather specialized equipment and procedures. On the other hand, the versatility of the fabrication process is such that impression dies for lagoons of practically any desired shapes and dimensions, and complexity of array can be produced as simply as that for a single circular lagoon. The process described was initially developed to provide a means whereby micro lagoon fields of any desired geometry could be readily impressed in common plastic culture vessels in numbers adequate to supply the particular research needs of the laboratory. For this purpose, the simple manual pressing arrangement described herein is adequate; however, the basic method should be readily adaptable to automated mass-production manufacture. In lieu of imprinting finished vessels, the die fabrication process can be used to produce surface molds having suitable lagoon-field patterns, and the fields can be directly cast in culture vessel surfaces at the time of vessel formation. Availability of such vessels in commercial quantities would be highly useful for a wide range of microbiological research purposes.

The primary advantages of the impressed lagoon fields, as compared with those formed by the grease-layer technique, may be summarized as follows:

(1) Any desired pattern of lagoon shapes, sizes, and array can be easily impressed into a wide range of plastic materials.

(2) The lagoon fields can be readily and permanently impressed into the surfaces of a range of presently existing plastic culture ware.

(3) Large numbers of culture vessels can be quickly imprinted, even with the manual techniques presently used. Adaptation to commercial mass production can be easily accomplished.

(4) Particular geometrical arrays of lagoons can be imprinted which permit simple and rapid location and identification of specific lagoons.

(5) The imprinted vessels may be easily sterilized after imprinting by either absolute ethanol or ethylene dioxide techniques, depending upon plastic compatibilities.

(6) The impressed lagoon shape and size can be precisely tailored to fit particular microscope observational requirements, such as in providing confinement for time-lapse fields without sacrifice of free test area.

(7) The depth of the imprinted lagoons can be accurately regulated.

(8) The impressed lagoons are rigid and stable, and thus are suitable for micromanipulation work directly in the lagoon.

Although the grease-layer technique for lagoon-field formation is highly useful for many laboratory purposes, the preceding list shows that impressed lagoon fields (when suitable facilities for their production are available) can provide a number of valuable extensions to the basic method, as well as new applications for specialized requirements.

Langley Research Center,
National Aeronautics and Space Administration,
Langley Station, Hampton, Va., March 20, 1969,
129-02-05-05-23.

REFERENCES

1. Cone, C. D., Jr.: Growth of Somatic Cell Clones in Micro Lagoons. Exp. Cell Res., vol. 48, no. 1, Oct. 1967, pp. 212-216.

2. Cone, Clarence D., Jr.; and Peddrew, Kathryn H.: A Micro Lagoon Technique for the Culture and Observation of Isolated Mammalian Cells. NASA TN D-4906, 1968.

3. Frank, Karl; and Becker, Mary C.: Microelectrodes for Recording and Stimulation. Electrophysical Methods, Part A. Vol. V of Physical Techniques in Biological Research, William L. Nastuik, eds., Academic Press, c.1964, pp. 22-87.

4. Cone, Clarence D., Jr.; and Tongier, Max, Jr.: Mitotic Synchronization of L-Strain Fibroblasts With 5-Aminouracil as Determined by Time-Lapse Cinephotography. NASA TN D-5021, 1969.

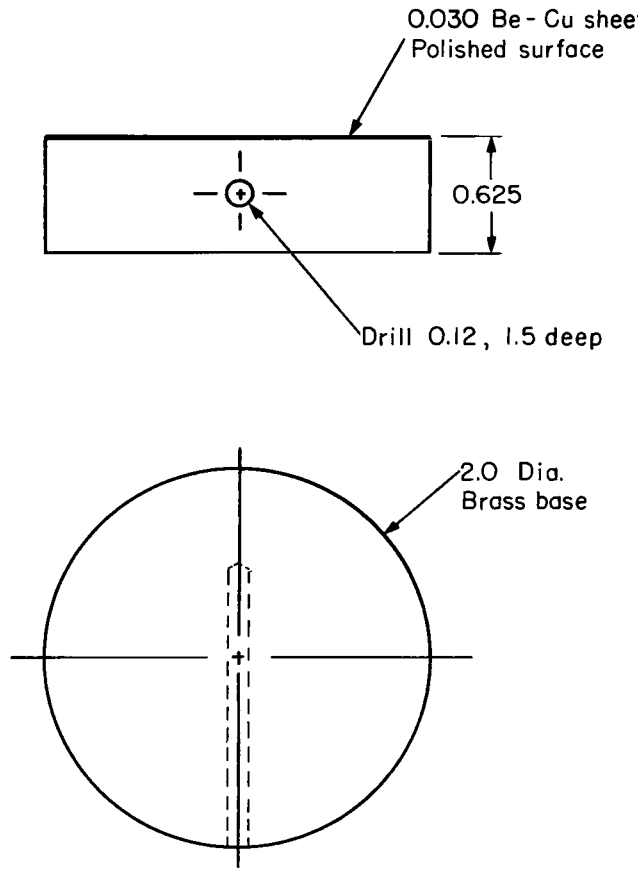

Figure 1.- Dimensions of a typical die image blank. All dimensions are in inches.

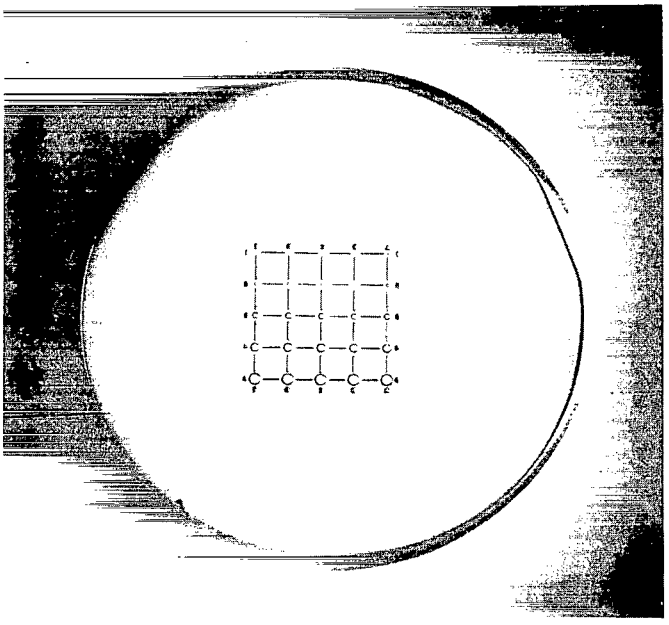

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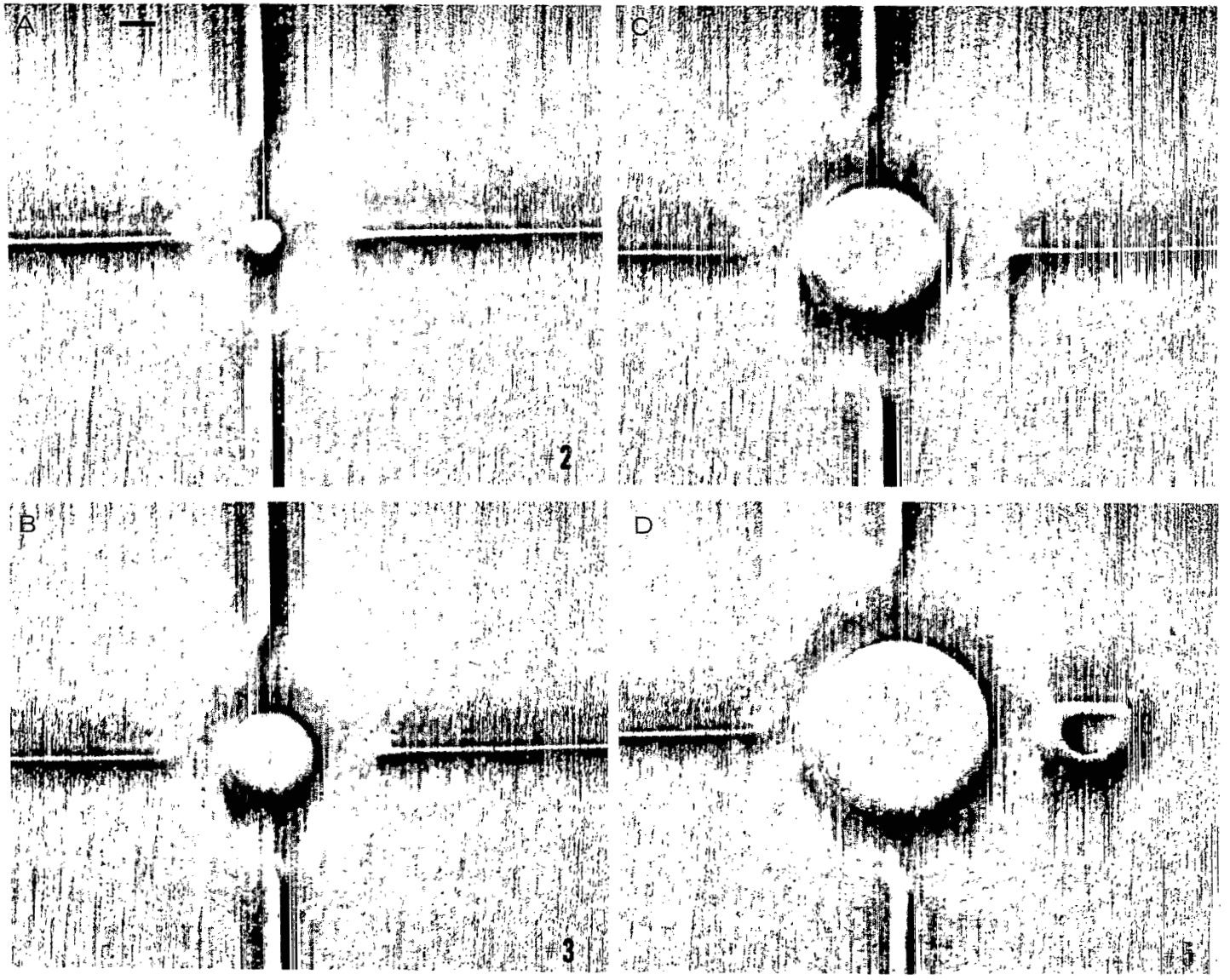
Figure 2.- Photograph of a finished die having a square array of lagoons. The raised image anvil of the lagoon array is clearly evident. Calibration: 1/4 inch.



L-69-1329

Figure 3.- Photomicrographs of representative lagoon spikes of the die shown in figure 2. The number in the lower right corner of each photograph identifies the lagoon row of the spike. Spikes are 45μ high. Calibration: 200μ.

A Diameter of photographic image.

B Diameter of actual lagoon spike.

h Spike height

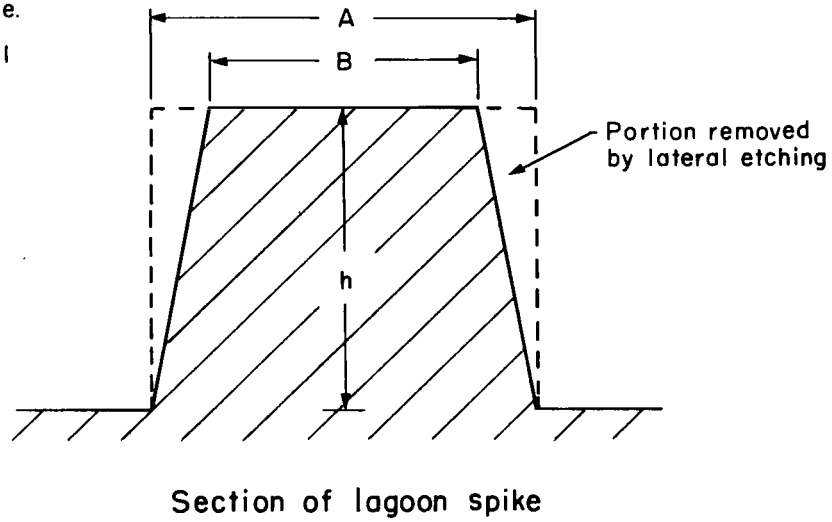


Figure 4.- Section view of a lagoon spike, showing how lateral removal of metal by chemical etching results in a conical form for the spike.

A Diameter of photographic image needed to get actual spike diameter B.

B Actual spike diameter.

C Diameter of spike if image of diameter B were used.

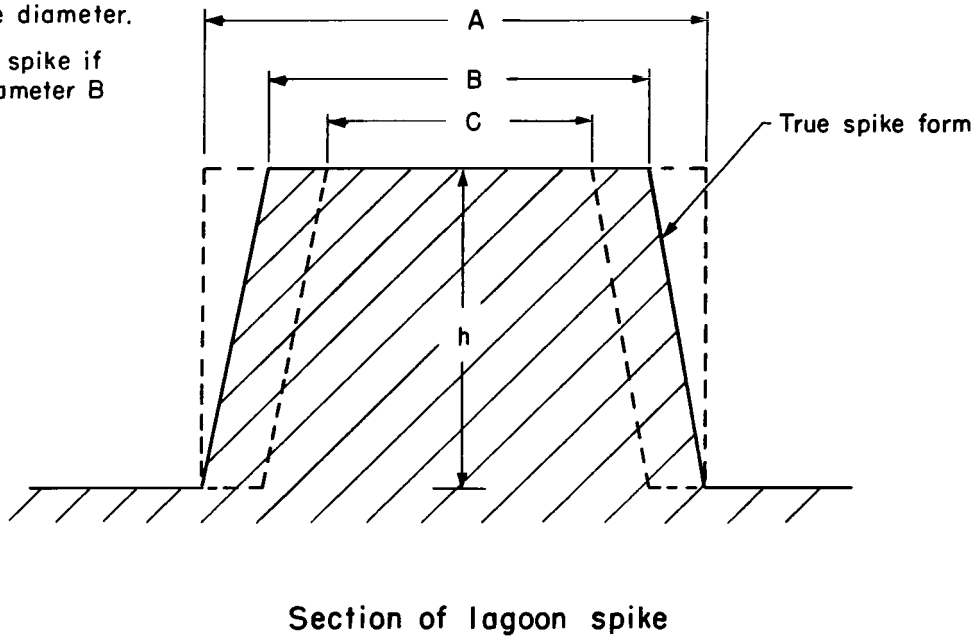
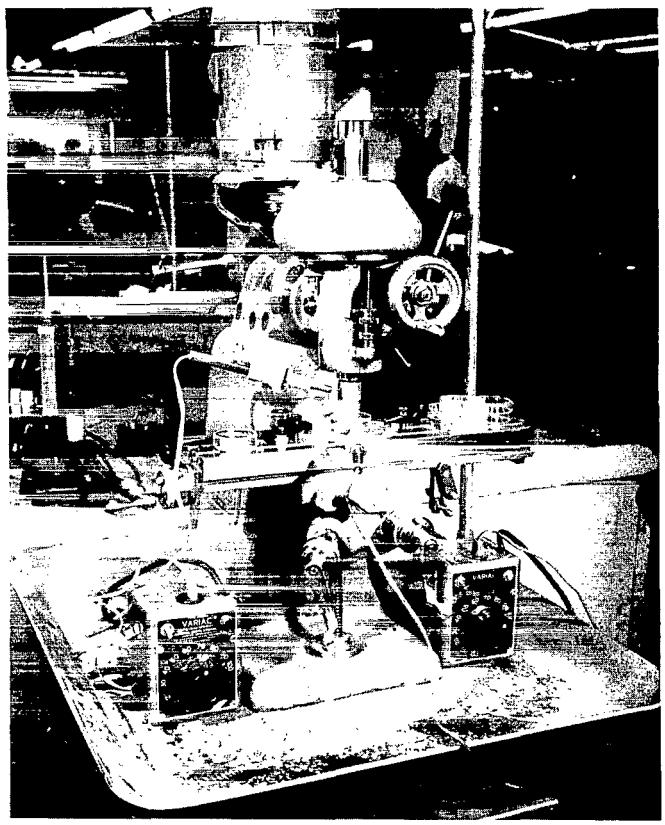
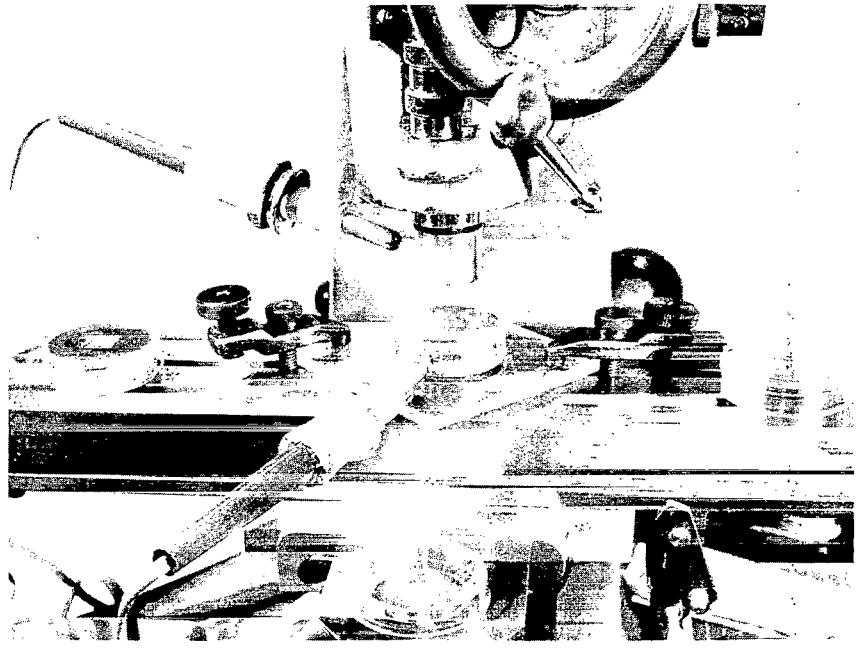


Figure 5.- Section view of a lagoon spike, showing how oversizing of image can result in attainment of desired spike size.



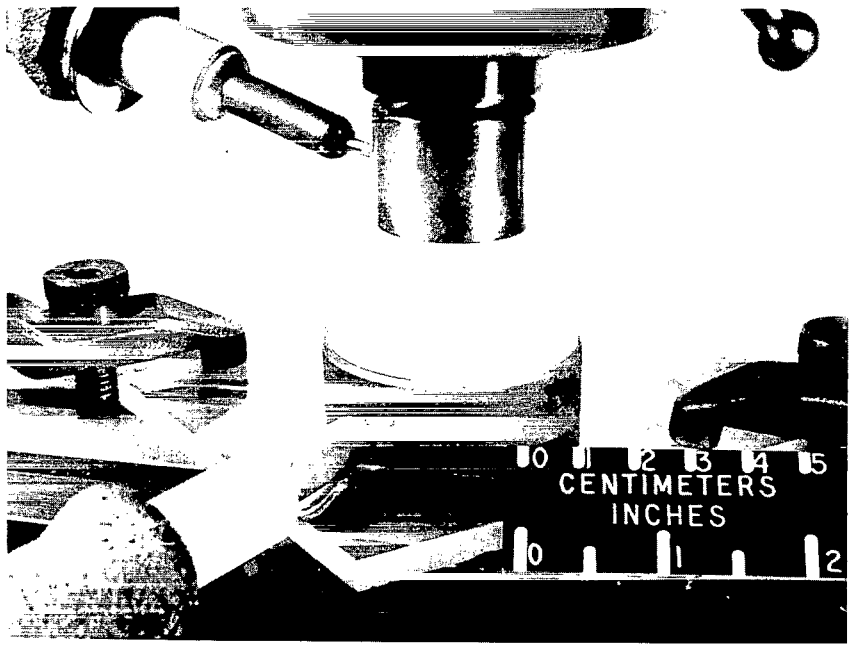
L-69-1330

Figure 6.- Photograph of general setup for impression of micro lagoon fields in plastic surfaces.



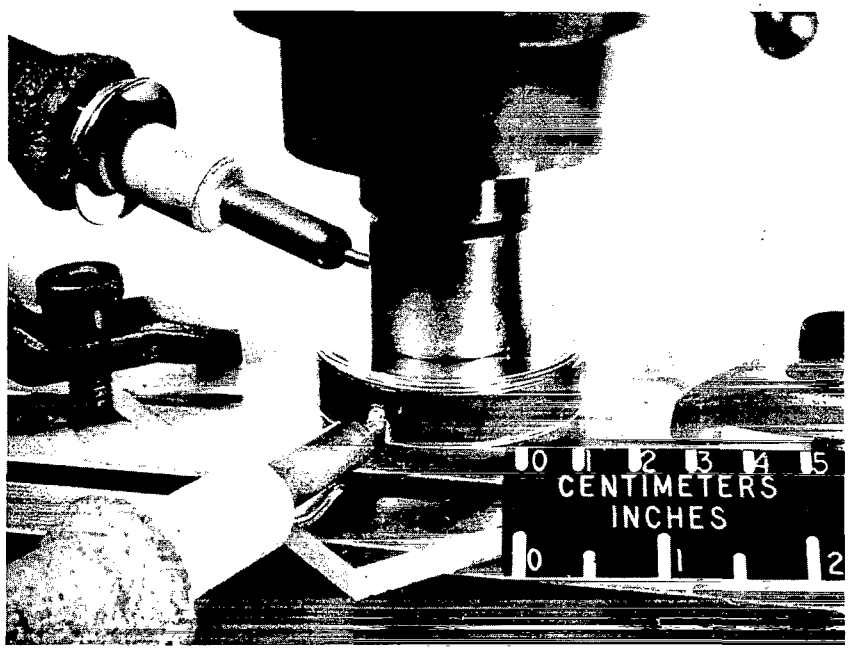
L-69-1331

Figure 7.- Closeup view of the impression setup. The heated press plate is mounted on the milling machine spindle.



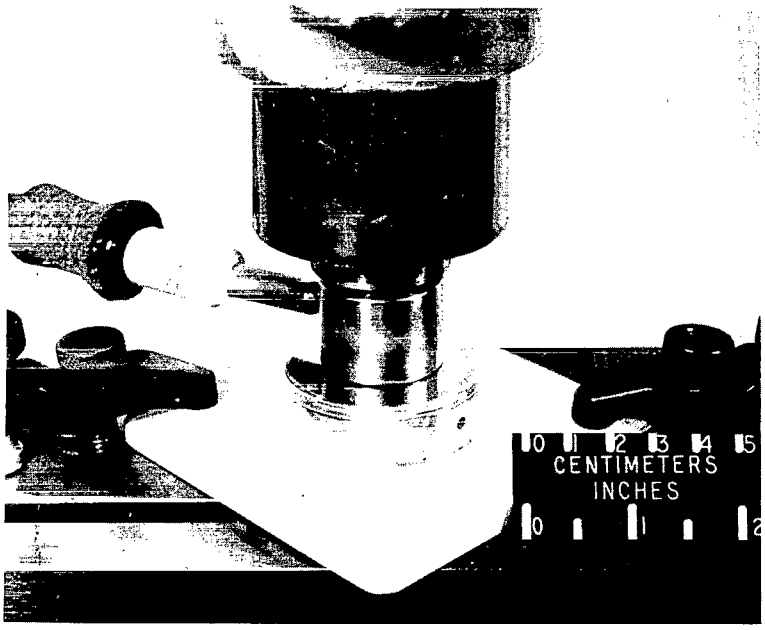
L-69-1332

Figure 8.- Closeup view of impression setup showing method for heating the impression die and press plate.



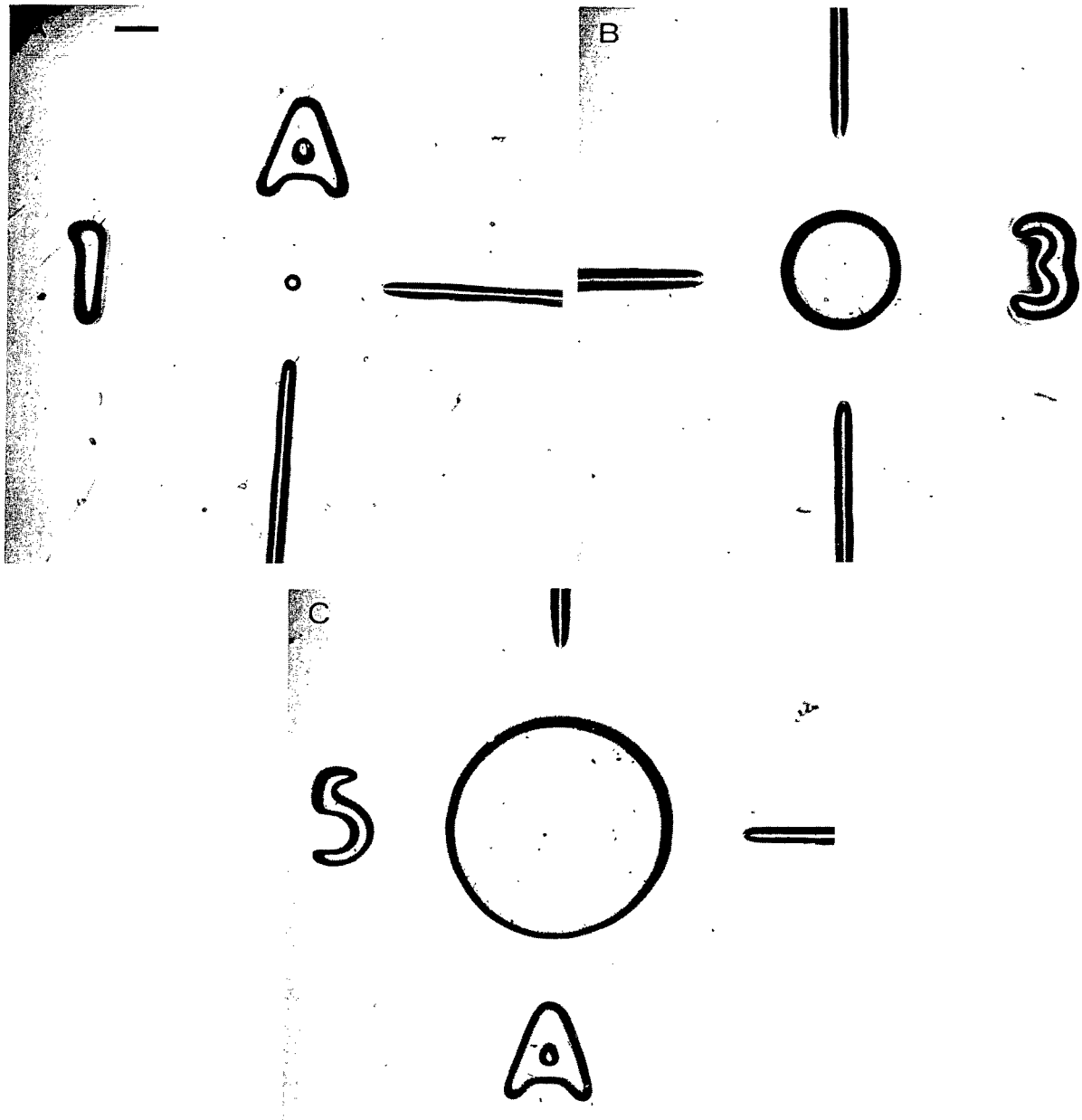
L-69-1333

Figure 9.- View of a plastic Petri dish bottom being impressed with a micro lagoon field. The dish bottom will be removed and used as a perfusion chamber window.



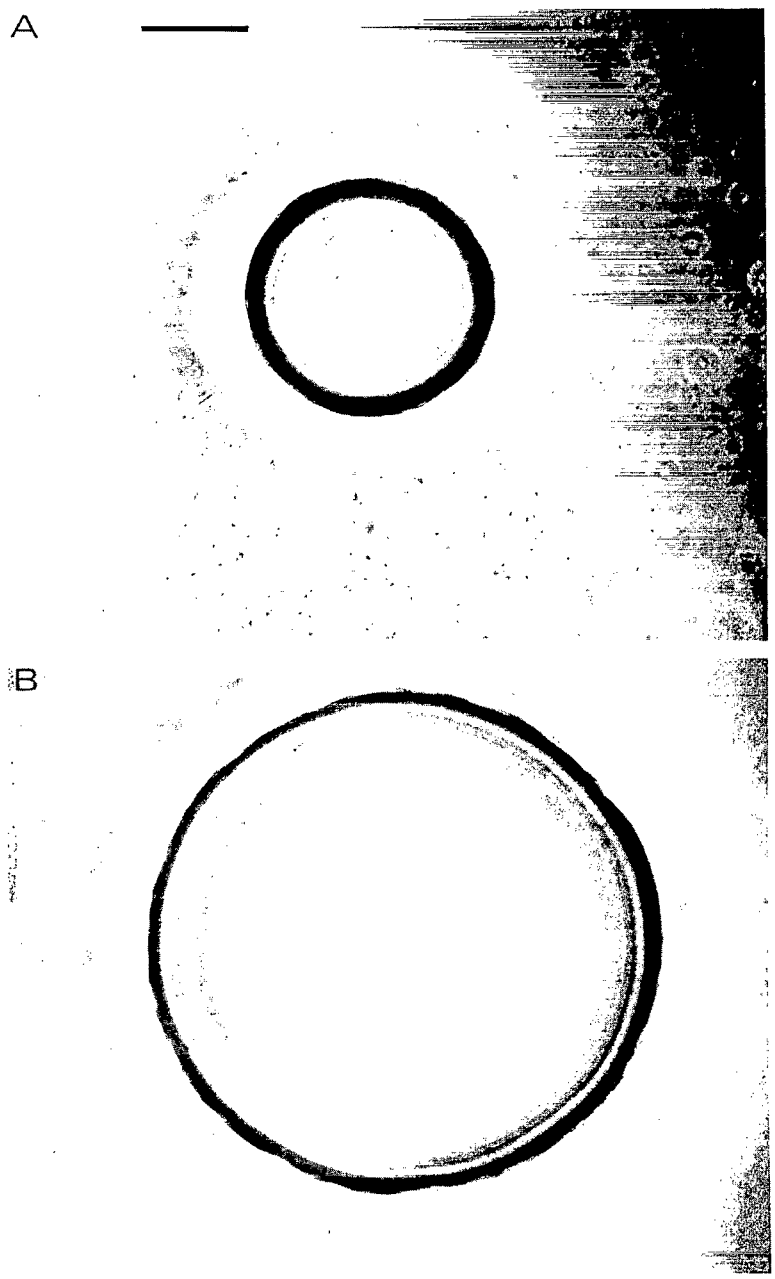
L-69-1334

Figure 10.- View of a plastic Petri dish being imprinted with a micro lagoon pattern for use with an inverted microscope.



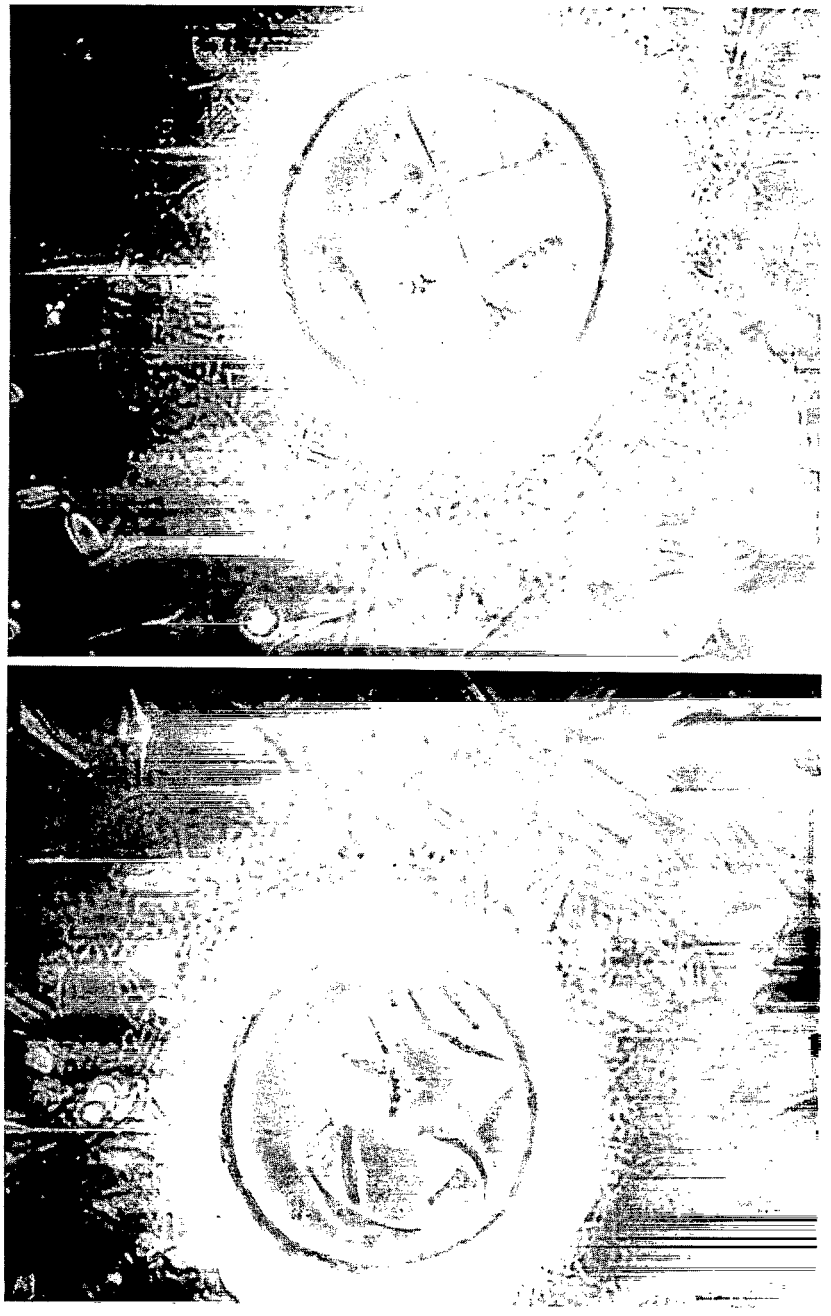
L-69-1335

Figure 11.- Low magnification photomicrographs of representative lagoons made with the impression die shown in figure 2. Calibration: 200μ.



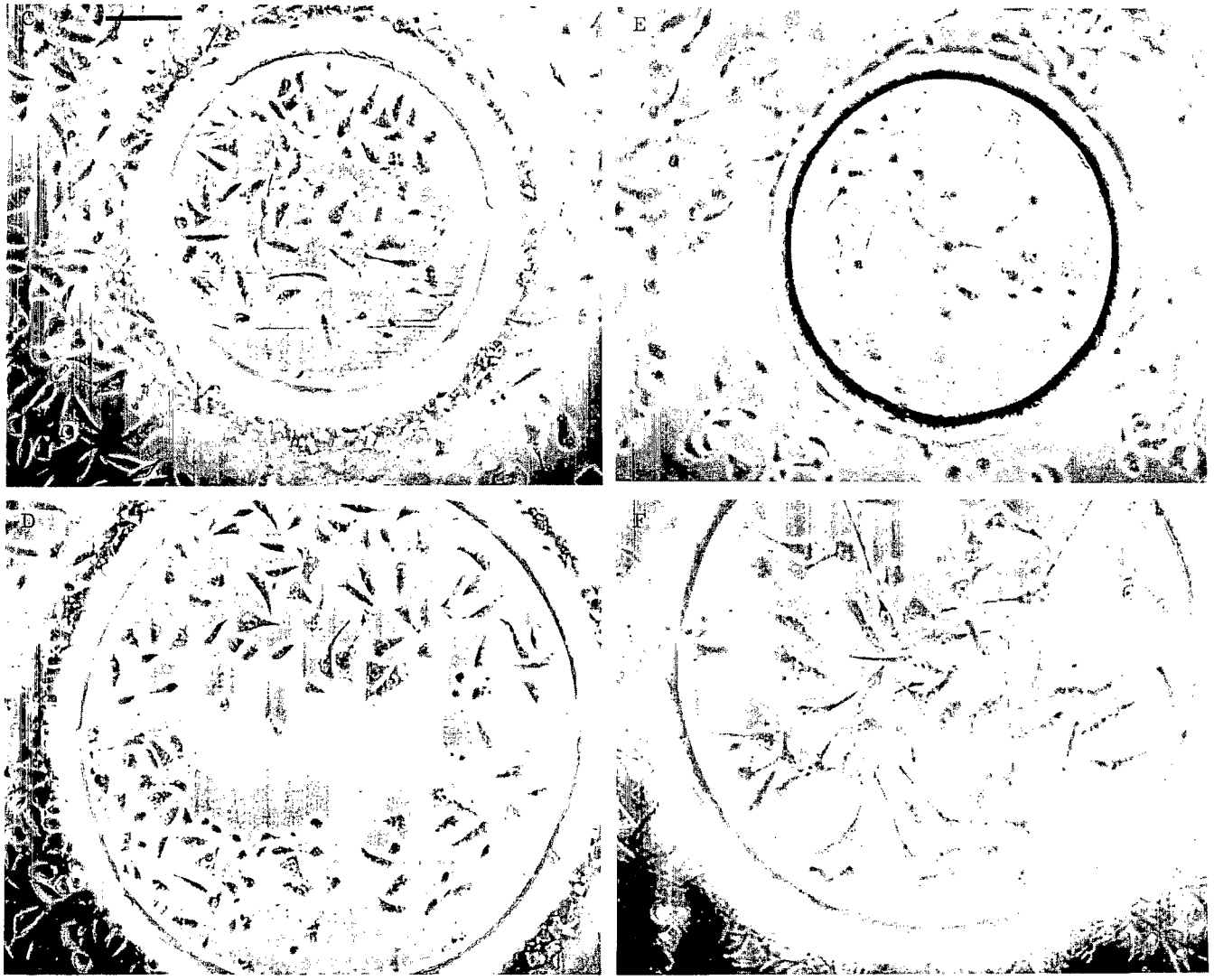
L-69-1336

Figure 12.- High magnification photomicrographs of two representative micro lagoons made with the impression die shown in figure 2. The lagoon shown in A is from row 2 and that in B from row 4. Calibration: 100μ.



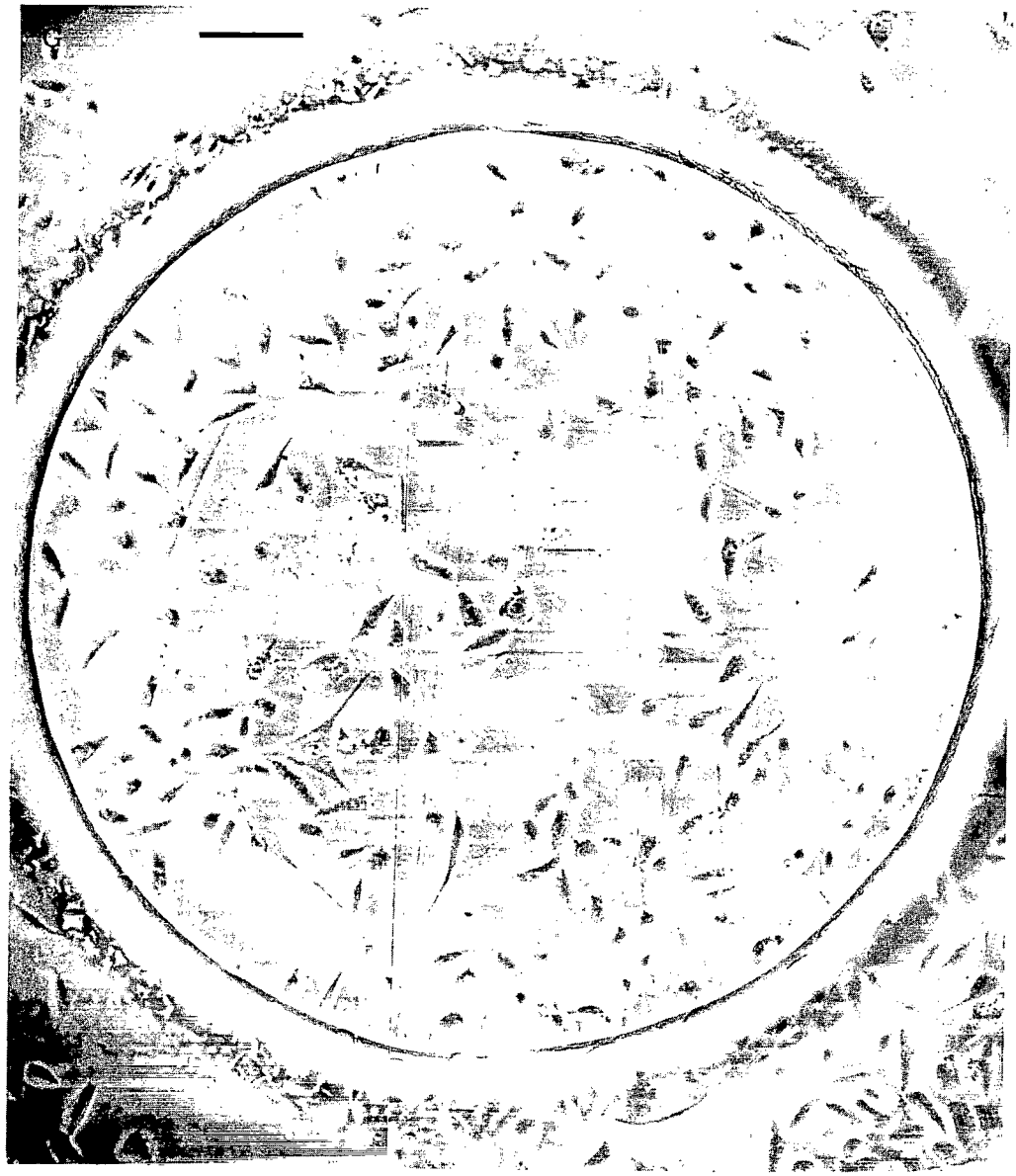
(a) Row 2 lagoons. Calibration: 65μ. L-69-1337

Figure 13.- Photomicrographs of typical lagoons containing L-strain mouse fibroblasts. Lagoons made with die shown in figure 2.



(b) C,E row 3 lagoons; D,F row 4 lagoons. Calibration (all lagoons): 100μ. L-69-1338

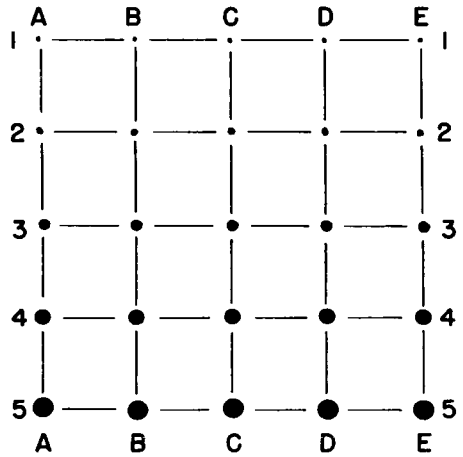
Figure 13.- Continued.



(c) Row 5 lagoon. Calibration: 100μ. L-69-1339

Figure 13.- Concluded.

Lagoon Sizes	
Row No.	Size, μ
1	100
2	200
3	500
4	750
5	1000



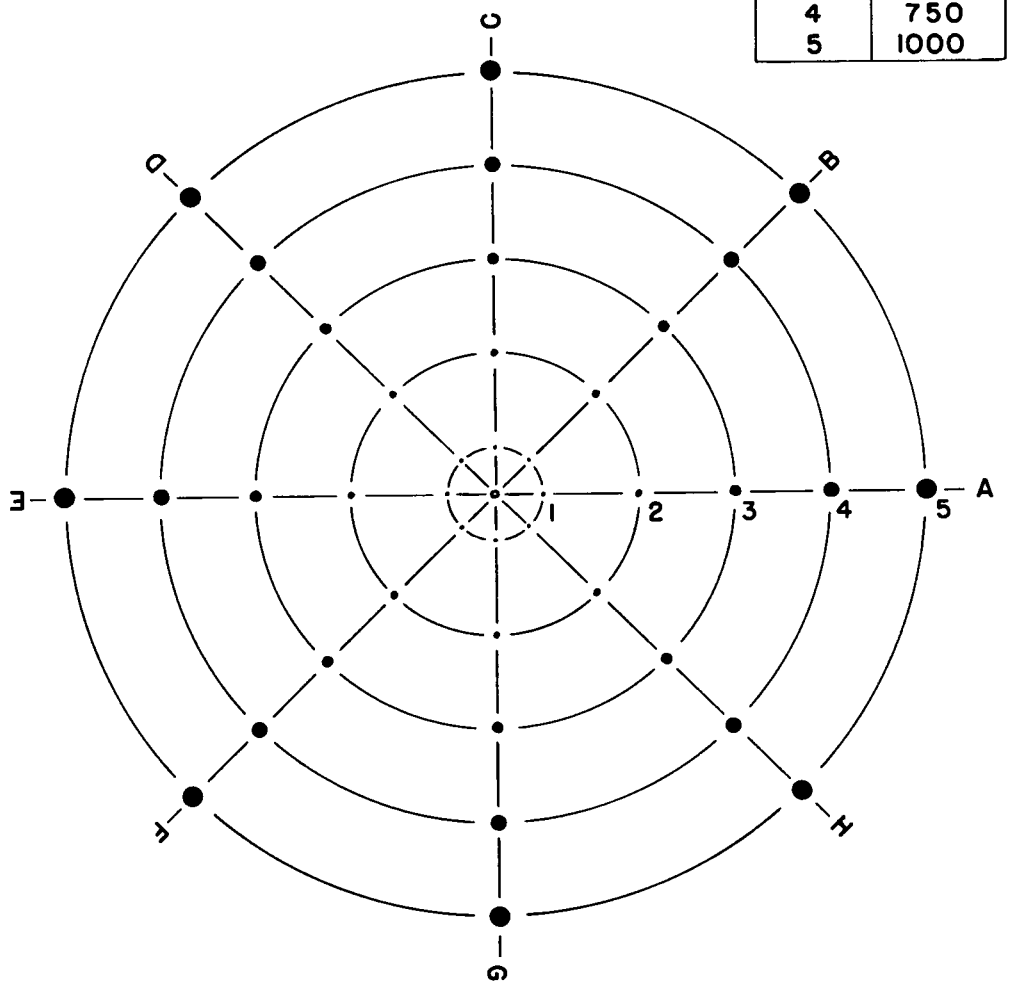
Square Array

A

(a) Square array.

Figure 14.- Useful lagoon field patterns. Actual patterns 1/2 size shown.

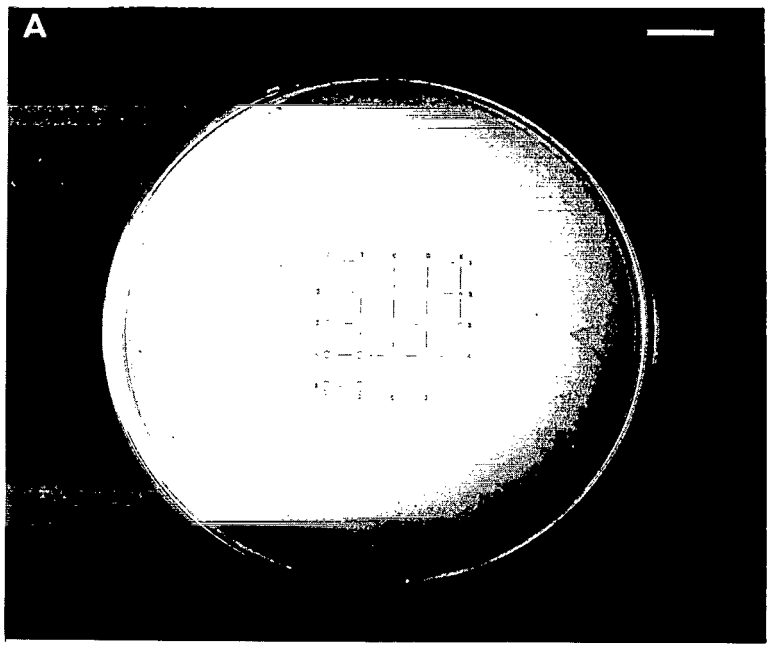
Lagoon Sizes	
Row No.	Size, μ
1	100
2	200
3	500
4	750
5	1000



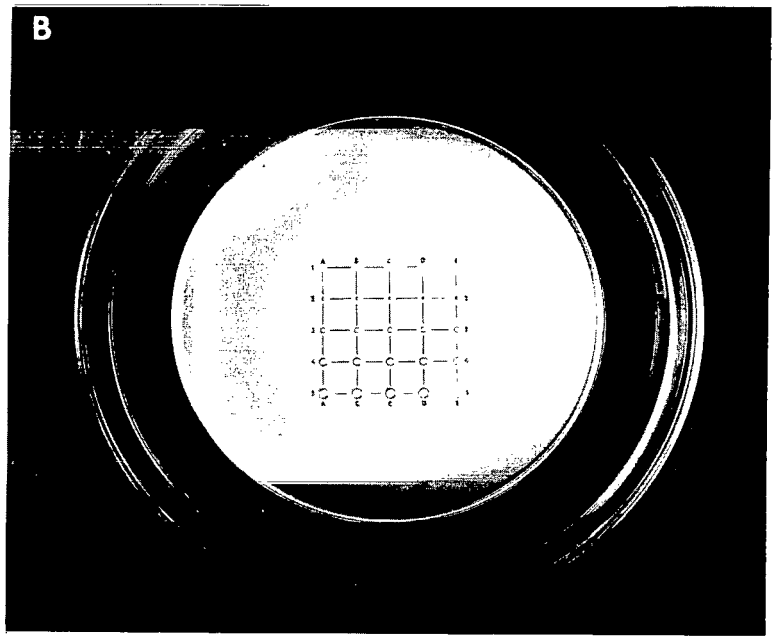
Circular Array
B

(b) Circular array.

Figure 14.- Concluded.



(a) Petri dish bottom.



(b) Cooper dish cover. L-69-1340

Figure 15.- Culture vessels imprinted with a square array of micro lagoons. Calibration: 1/4 inch.

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